UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/492,590	01/27/00	CARTENS C	41114/85530

HM22/0809

Kathleen M Williams Esq
Banner & Witcoff LTD
28 State Street 28th Floor
Boston MA 02109

EXAMINER	
LEFFERS JR, G	
ART UNIT	**PAPER NUMBER**
1636	8

DATE MAILED: 08/09/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/492,590	Applicant(s) Carstens, Carsten-Peter
	Examiner Gerald G. Leffers Jr.	Group Art Unit 1636

☐ Responsive to communication(s) filed on _____ .

☐ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-44* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-44* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Acknowledgment is made of applicant's claim for priority under 35 U.S.C. 119(e) to U.S. Provisional Application 60/117,355.

Claim Objections

Claim 35 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 33 is drawn to a method of producing a protein of interest by culturing the host cells under conditions "wherein the conditions of culturing said host cell are sufficient to produce said protein of interest". Claim 35 is dependent on claim 33 and appears to be redundant in that it is drawn to "further comprising the step of producing said protein of interest.".

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 17 and 20 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "Hte (high transformation efficiency) phenotype" does not appear to be well defined in the specification, making the metes and bounds of the claim indefinite. What transformation frequency would constitute an "Hte" phenotype for a competent cell? Would a frequency of transformation of ~10^5 transformants per ug of plasmid DNA qualify a competent cell preparation as having an "Hte" phenotype? If so, under what transformation conditions (e.g. $CaCl_2$ transformation or electroporation) would this phenotype be determined? It would be remedial to amend the claim language to clearly indicate what is intended by the limitation of an "Hte" phenotype.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-5, 10-17, 22-23 and 26-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Del Tito et al (U) in view of Makoff et al (V).

Del Tito et al teach the construction and use of a plasmid, pRI952, which comprises an array of two tRNA genes (argU and ileX) encoding tRNAs specific for the rarely used codons AGG/AGA and AUA, respectively (page 7087, paragraph 2; Tables I and II). The authors teach

that pRI952 was constructed by insertion of a PCR-amplified DNA comprising the gene for ileX flanked by HindIII restriction sites into pDC592, a pACYC184 derivative (i.e. low copy number) already possessing the argU gene (page 7087, column 2, paragraph 2). Del Tito et al teach that coexpression of the two tRNA genes along with the gene encoding the heterologous polypeptide Mup^r IRS results in increased levels of active protein as compared to a control in which no additional tRNA genes are expressed or as compared to cells comprising a plasmid only expressing the ileX gene (Table II). Del Tito et al teach that "..problems in expression can be avoided by a careful inspection of the coding sequence and inclusion of appropriate tRNA genes or necessary site-specific mutations." (page 7087, column 1, paragraph 2). The authors conclude that the coexpression of minor tRNAs such as ileX or argU can be utilized to overcome translational stresses due to the presence of rarely used codons within the coding sequence for a gene of interest (page 7091, column 1, paragraph 3). Del Tito et al teach the purification by reverse phase HPLC of another heterologous polypeptide (i.e. the B/LeeHA antigen) produced by their system for compensating for the presence of rare codons in the coding sequence for the desired polypeptide (page 7088, column 1, paragraphs 3-4).

Del Tito et al do not explicitly teach the use of a vector comprising an array of 3 or more tRNAs corresponding to rarely used codons for overexpression of a heterologous gene comprising rarely used codons. Del Tito et al do not explicitly teach the use of ileY, proL, leuW.

Makoff et al teach that the expression of the tetanus toxin fragment C in E.coli is limited by its high demand for rare tRNA molecules (page 10193, paragraph 2). Makoff et al teach that

fragment C comprises several different rare codons specifying different amino acids (i.e. Leu, Ile, Ser, Pro, Arg and Gly) which are fairly evenly spread out through the coding sequence (Table 2; page 10196, paragraph 2). Makoff et al teach that replacement of almost the entire coding sequence with synthetic sequence which lacks the rarely used codons results in an approximate 4-fold increase in expression of the desired heterologous polypeptide (page 10199, paragraph 2). Makoff et al teach that fragment C from tetanus toxin shows considerable promise as a subunit vaccine against tetanus (page 10193, paragraph 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the vector taught by Del Tito comprising argU and ileX for increasing the expression of a desired heterologous polypeptide whose gene comprises rarely used codons by introducing additional tRNA genes corresponding to rarely used codons other than AGA, AGG or AUA in order to express the tetanus fragment C subunit in E.coli as taught by Makoff et al because Del Tito et al teach that it is within the skill of the art to express tRNA genes corresponding to different rarely used codons from the same vector in order to compensate for the presence of the rarely used codons in a gene encoding a desired heterologous polypeptide, because Makoff et al teach it is within the skill of the art to increase the expression of fragment C in E.coli by compensating for the presence of a number of different rarely used codons in the gene encoding fragment C and because tRNA genes corresponding to the rarely used codons in the gene encoding tetanus toxin fragment C are and were known in the art. One would have been motivated to do so in order to receive the expected benefit of expressing increased levels of

fragment C from the native gene encoding fragment C without having to synthetically construct a gene encoding fragment C which lacks the rarely used codons. Based upon the combined teachings above, and absent any evidence to the contrary, there would have been a reasonable expectation of success in utilizing a vector made from the combined teachings above comprising 3 or more tRNA genes corresponding to the rare codons present in the coding sequence for fragment C, as taught by Makoff et al, to overexpress fragment C from its native gene in E.coli.

With regard to the different tRNA genes recited in the rejected claims (e.g. ileY, proL or leuW), it would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate any tRNA gene known in the art into the vector made from the combined teachings above in order to provide the necessary tRNAs to compensate, as taught by Del Tito et al, for the presence of the rarely used codons present in the fragment C gene, as taught by Makoff et al. Absent any evidence to the contrary, there would have been a reasonable expectation of success in utilizing such tRNA genes in the expression system made from the combined teachings above to increase the expression of fragment C in E.coli.

Claims 6-9, 19, 21 and 24-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Del Tito et al (U) in view of Makoff et al (V) as applied to claims 1-5, 10-17, 22-23 and 26-38 above, and further in view of the 1997 Novagen catalog (pages 42-44) (W).

The teachings of Del Tito et al and Makoff et al are described above and applied as before, except:

Del Tito et al teaches that the expression of tRNA genes has been shown to be deleterious to the host cell and that for this reason the ileX promoter was used to control expression of the ileX gene from low-copy number plasmids (page 7090, column 2, paragraph 3).

Neither reference teaches the use of a vector in which the expression of the tRNA genes is regulated by an IPTG inducible promoter, the use of a T7 RNA polymerase promoter or protease deficient cells.

The 1997 Novagen catalog (pages 42-44; Figure 1) describes a T7 RNA polymerase expression system for tight control over the expression of toxic genes in E.coli. The system features 1) the use of a λ lysogen (DE3) which comprises the gene for T7 RNA polymerase under control of an IPTG-inducible promoter, 2) a T7lac promoter which is also inducible upon addition of IPTG and (page 44) and 3) an E.coli strain which lacks functional genes for the Lon and OmpT proteases (page 43, paragraph 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to clone the tRNA genes for tRNAs corresponding to rarely used codons used in the methods made from the combined teachings above into one of the pET vectors/expression systems described in the 1997 Novagen catalog because Del Tito et al teach that it is within the skill of the art to compensate for the presence of rarely used codons in the gene for a polypeptide of interest by expressing the corresponding tRNA genes from a vector in E.coli, because Del Tito et al also teach that the expression of tRNA genes in E.coli can have negative effects on the host cells and because the T7 RNA polymerase-based system described in the Novagen catalog for

tightly controlled expression of target, toxic genes in E.coli was well known and widely used within the art for the expression of toxic genes in E.coli. One would have been motivated to do so in order to avoid any potential toxic effects associated with the expression of the tRNA genes in E.coli. Absent any evidence to the contrary, there would have been a reasonable expectation of success in utilizing the pET expression system for the controlled expression of tRNA genes in E.coli for the purposes of expression desired polypeptides whose genes comprise a number of different, rarely used codons.

Claims 18 and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Del Tito et al (U) in view of Makoff et al (V) and the 1997 Novagen catalog (W) as applied to claims 1-17, 19 and 21-38 above, and further in view of Wnendt (X).

The teachings of Del Tito et al, Makoff et al and the 1997 Novagen catalog are described above and applied as before, except:

The cited references do not teach the use of endA$^-$ E.coli strains.

Wnendt teaches that the use of endA$^-$ strains allows for great and higher quality yields of plasmid DNAs from bacterial cells (page 270, column 1, paragraph 1).

It would have been obvious to one of ordinary skill in the art to introduce an endA mutation into the strain of E.coli used in the method made from the combined teachings above to express heterologous polypeptides whose genes comprise different rarely-used codons because Wnendt teaches that the lack of EndA nuclease activity results in higher quantity and quality of

plasmids isolated from E.coli strains bearing an endA mutation. One would have been motivated to do so in order to increase the yield and quality of plasmids recovered from the host cells during cloning of the vectors comprising the tRNA genes corresponding to the rarely used codons present in the gene encoding a desired polypeptide. Absent any evidence to the contrary, there would have been a reasonable expectation of success in utilizing an endA$^-$ strain of E.coli in the methods made from the combined teachings above for expression of a gene encoding a desired polypeptide comprising rarely used codons.

Claims 39-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Del Tito et al (U).

Del Tito et al teach the construction and use of a plasmid, pRI952, which comprises an array of two tRNA genes (argU and ileX) encoding tRNAs specific for the rarely used codons AGG/AGA and AUA, respectively (page 7087, paragraph 2; Tables I and II). Del Tito et al teach that coexpression of the two tRNA genes along with the gene encoding the heterologous polypeptide Mup^r IRS results in increased levels of active protein as compared to a control in which no additional tRNA genes are expressed or as compared to cells comprising a plasmid only expressing the ileX gene (Table II). Del Tito et al teach that "..problems in expression can be avoided by a careful inspection of the coding sequence and inclusion of appropriate tRNA genes or necessary site-specific mutations." (page 7087, column 1, paragraph 2). The authors conclude that the coexpression of minor tRNAs such as ileX or argU can be utilized to overcome

translational stresses due to the presence of rarely used codons within the coding sequence for a gene of interest (page 7091, column 1, paragraph 3).

Del Tito et al do not explicitly teach the use of any vector nucleic acid comprising two tRNA genes corresponding to rarely used codons other than a vector comprising argU and ileX. Del Tito et al do not teach the use of ileY, proL , leuW or a tRNA corresponding to rarely used glycine codons.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the vector construct taught by Del Tito et al for compensating for the presence of rarely used codons present in the gene encoding a polypeptide of interest by interchanging different tRNA genes corresponding to other rarely used codons (i.e. other than AGG/AGA or AUA) because Del Tito et al teach that it is within the skill of the art to carefully scrutinize the coding sequence for a desired polypeptide, identify rarely used codons and compensate for the presence of such rarely used codons by supplying in trans the tRNA corresponding to the identified rarely used codons from a vector expressing different tRNA genes, and because such rarely used codons and the genes for their corresponding tRNAs are and were known in the art. One would have been motivated to do so in order to meet the particular rare-codon requirements of a gene encoding a desired polypeptide and thus receive the expected benefit of increasing its expression in E.coli, as taught by Del Tito et al. Absent any evidence to the contrary, there would have been a reasonable expectation of success in utilizing any tRNA gene known in the art

(i.e. ileY, proL, leuW, etc.) in the vector taught by Del Tito et al to increase the production of a desired polypeptide in E.coli whose gene comprises different rarely used codons.

Conclusion

No claims are allowed.

Certain papers related to this application may be submitted to Art Unit 1636 by facsimile transmission. The faxing of such papers must conform with the notices published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 C.F.R. § 1.6(d)). The official fax telephone numbers for the Group are (703) 308-4242 and (703) 305-3014. NOTE: If Applicant *does* submit a paper by fax, the original signed copy should be retained by applicant or applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED so as to avoid the processing of duplicate papers in the Office.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gerald Leffers, Jr. whose telephone number is (703) 308-6232. The examiner can normally be reached on Monday through Friday, from about 9:00 AM to about 5:30 PM. A phone message left at this number will be responded to as soon as possible (usually no later than 24 hours after receipt by the examiner).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. George Elliott, can be reached on (703) 308-4003.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

GGL

G. Leffers, Jr.

Patent Examiner

Art Unit 1636

DAVID GUZO
PRIMARY EXAMINER

August 4, 2000